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COMMENT

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Integrated Mendelian randomization and single-cell RNA-sequencing analyses identified OAS1 as a novel therapeutic target for erectile dysfunction via targeting fibroblasts

Yi Wang[1,2†], Guihua Chen[2†] and Deng Li[2*]

Abstract

Clinically, phosphodiesterase type 5 inhibitors (PDE5-Is) remain the first-line therapy for erectile dysfunction (ED) patients; however, approximately 35% of these patients are still failing to respond to the therapeutic effects. So, urgent needs are required to identify novel therapeutic targets for ED. Hence, in this report, it was the first time for us to integrate single-cell RNA-sequencing (scRNA-Seq), mendelian randomization (MR) analysis with expression quantitative trait loci (eQTL), and protein quantitative trait loci (pQTL) data to find new treatment targets for ED. Disease-causing changes were revealed by MR analysis, and it showed that the OAS1 eQTL/cis-eQTL/cis-pQTL was causally related to ED, significantly reducing its risks (all $P < 0.05$). Disease-induced changes were revealed by scRNA-Seq, and it suggested that OAS1 mainly played its role in ED via targeting fibroblasts. We further concluded that the positive regulation of OAS1 gene expression could lead to the vicious circle of ED. As a result, drugs targeting OAS1 in the future might provide more potential opportunities and flexibility for treating ED. In conclusion, our study identified OAS1 as a gene of interest in the context of ED via targeting fibroblasts through integrated MR and scRNA-Seq analyses. While these findings highlighted the potential of OAS1 as a therapeutic target, further experimental and clinical studies were still required to validate its functional role and therapeutic relevance in ED pathology.

Keywords Mendelian randomization, OAS1, Single-cell RNA-sequencing, Erectile dysfunction, Fibroblasts

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Dear Editor

Clinically, phosphodiesterase type 5 inhibitors (PDE5-Is) remain the first-line therapy for erectile dysfunction (ED) patients; however, approximately 35% of these patients are still failing to respond to the therapeutic effects [1]. So, urgent needs are required to identify novel therapeutic targets for ED. Currently, mendelian randomization (MR) is a practical and effective technique that is being extensively used to seek potential drug targets for a range of diseases [2]. Therefore, in this article, we firstly integrated integrate single-cell RNA-sequencing (scRNA-Seq), MR analysis with expression quantitative trait loci (eQTL) and protein quantitative trait loci (pQTL) data to find new treatment targets for ED.

A total of four independent datasets were enrolled as exposures in our analysis, containing the discovering dataset (IEU OpenGWAS eQTLs) and three validating datasets (eQTLGen Whole Blood cis-eQTLs, GTEx Whole Blood cis-eQTLs, and Zheng Plasma Protein cis-pQTLs). Therein, IEU OpenGWAS eQTLs were from the website link https://gwas.mrcieu.ac.uk/; eQTLGen Whole Blood cis-eQTLs were from the website link https://www.eqtlgen.org/cis-eqtls.html; GTEx Whole Blood cis-eQTLs were from the website link https://yanglab.westlake.edu.cn/software/smr/#eQTLsummarydata; and Zheng Plasma Protein cis-pQTLs were from the article of Zheng et al. [3]. As for outcomes, ED genetic data were obtained from the website link https://gwas.mrcieu.ac.uk/, with IDs of ebi-a-GCST006956, including 6,175 cases and 217,630 controls. ScRNA-Seq data of ED was got from the GSE206528 dataset, including three normal and three ED patients' corpus cavernosum tissues [4].

For the causal effects of IEU OpenGWAS eQTLs on ED, instrumental variables (IVs) were selected based on the P-value threshold of 5e-8, defaulted linkage disequilibrium (LD) threshold, and F-statistic > 10 [5]; two-sample MR analysis was conducted, and the inverse variance weighting (IVW) P-values below 0.05 were regarded as statistically significant. For the causal effects of eQTLGen Whole Blood cis-eQTLs/GTEx Whole Blood cis-eQTLs on ED, summary-data-based MR (SMR) was conducted by the SMR v1.0.2 software with the defaulted options [6] in the command line, and the SMR P-values below 0.05 were regarded as statistically significant. For the causal effects of Zheng Plasma Protein cis-pQTLs on ED, IVs were provided in the article of Zheng et al. [3]; two-sample MR analysis was conducted, and the inverse variance weighting (IVW) or the weighted median P-values below 0.05 were regarded as statistically significant [7]. Sensitivity analysis for two-sample MR analysis was evaluated by pleiotropy and heterogeneity. Sensitivity analysis for SMR analysis was assessed by the heterogeneity in dependent instruments (HEIDI) test [6]. Data analyses of the GSE206528 scRNA-Seq dataset were conducted as detailed in the article [4]. Therein, cell clusters were conducted by the "FindClusters" function of the "Seurat" v 4.4.0 package and performed UMAP (Uniform Manifold Approximation and Projection) dimensionality reduction. The fibroblast cells were annotated by the markers "PDGFRA", "LUM", and "COL1A1".

Based on our analysis, OAS1 was identified as the only intersected gene for the causal effects of four independent datasets on ED (Fig. 1A). As detailed in Fig. 1B, the OAS1 eQTL was causally related to ED and could reduce its risks in the IEU OpenGWAS dataset (the IVW method, beta = −0.055, 95% confidence interval (CI) = −0.104 to −0.005, $P = 0.030$, $P_{pleiotropy}$ = pass, $P_{heterogeneity}$ = pass); the OAS1 cis-eQTL was causally

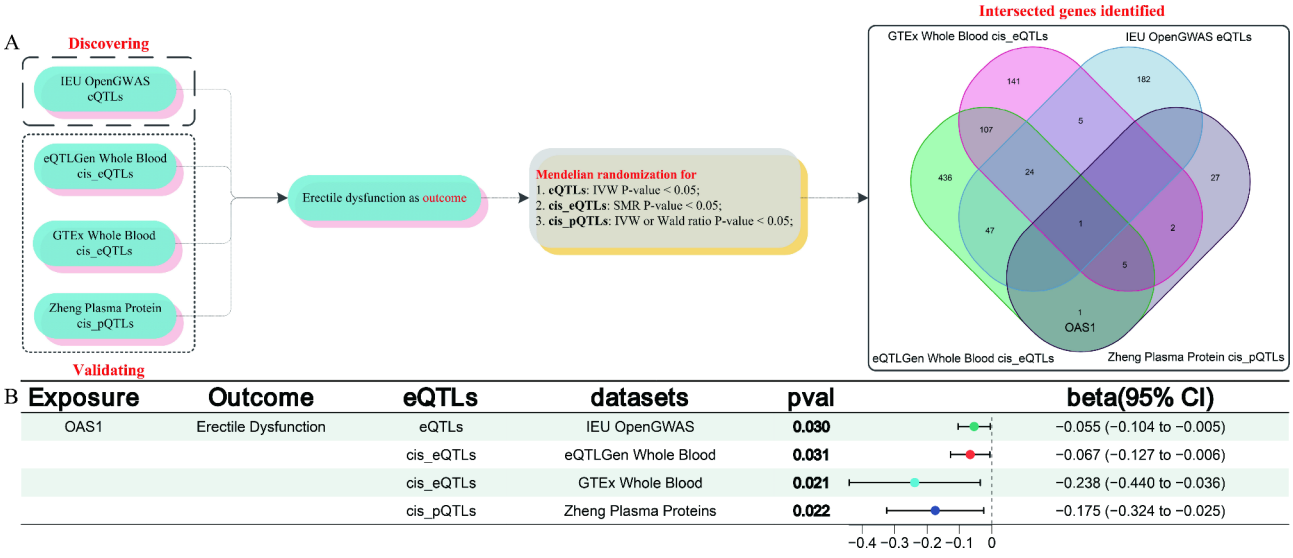

Fig. 1 The causal effects of OAS1 on ED; **(A)** Four independent datasets identified OAS1 as the only intersected gene causally linked with ED; **(B)** Detailed MR results of the causal effects of OAS1 on ED; Bold font means $P < 0.05$;

related to ED and could reduce its risks in the eQTLGen Whole Blood dataset (the SMR method, beta = – 0.067, 95% CI = – 0.127 to – 0.006, $P = 0.031$, $P_{HEIDI\ =}$ pass); the OAS1 cis-eQTL was causally related to ED and could reduce its risks in the GTEx Whole Blood dataset (the SMR method, beta = – 0.238, 95% CI = – 0.440 to – 0.036, $P = 0.021$, $P_{HEIDI\ =}$ pass); the OAS1 cis-pQTL was causally related to ED and could reduce its risks in the Zheng Plasma Protein cis-pQTLs dataset (the weighted median method, beta = – 0.175, 95% CI = – 0.324 to – 0.025, $P = 0.022$).

Results of scRNA-Seq analysis showed that OAS1 was mainly expressed in the cell cluster of fibroblasts by UMAP (Fig. 2A). Moreover, the expression levels of OAS1 in the fibroblasts cell cluster was significantly different from that in the endothelial cell, the smooth muscle cell and the dendritic cell clusters (all $P < 0.001$; Fig. 2B). As presented in Fig. 2C, the expression levels of OAS1 were significantly lower in all cell clusters in ED

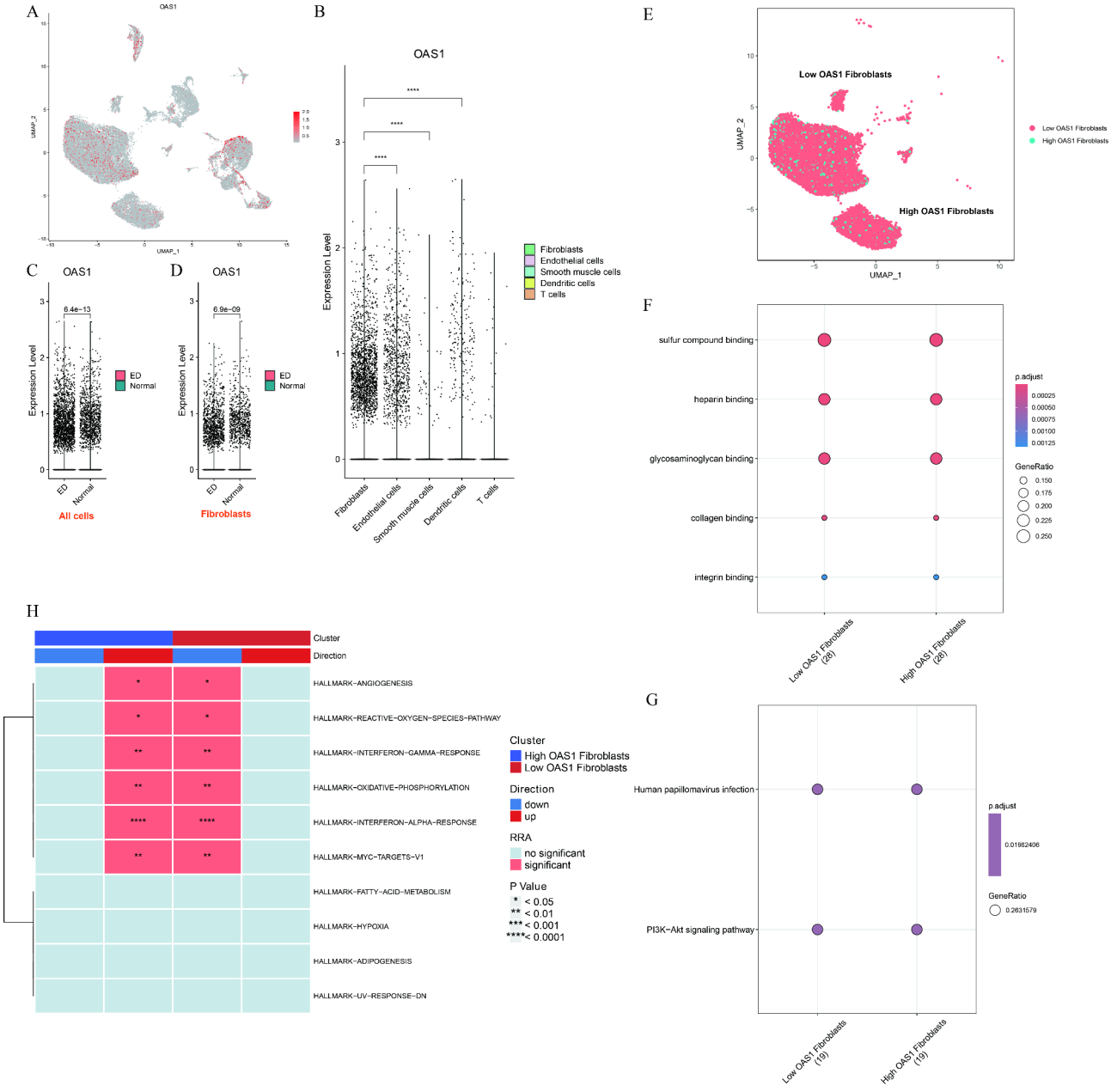

Fig. 2 Single-cell RNA-sequencing (scRNA-Seq) data analysis of OAS1 in ED; **(A)** The expression levels of OAS1 in ED by UMAP; **(B)** The expression differences of OAS1 in various cell clusters; **(C)** The expression differences of OAS1 in all cell clusters of ED and normal patients; **(D)** The expression differences of OAS1 in the fibroblasts cell cluster of ED and normal patients; **(E)** UMAP plot of low/high OAS1 fibroblasts; **(F)** GO analysis of low/high OAS1 fibroblasts; **(G)** KEGG analysis of low/high OAS1 fibroblasts; **(H)** irGSEA analysis of low/high OAS1 fibroblasts;

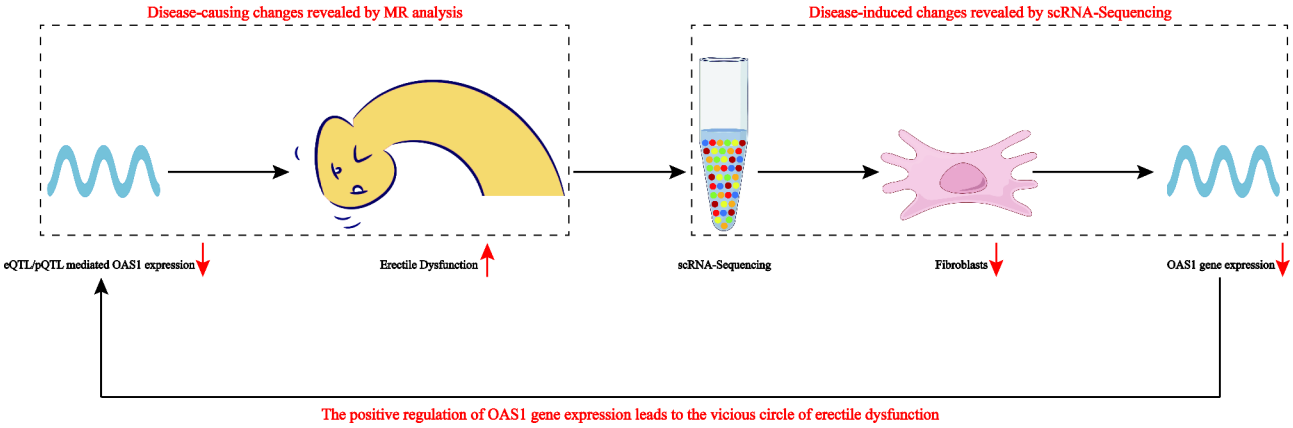

Fig. 3 Integrated MR and scRNA-Seq analyses revealed that the positive regulation of OAS1 gene expression could lead to the vicious circle of erectile dysfunction;

patients than in normal patients ($P<0.001$). Besides, the expression levels of OAS1 had a markedly lower expression in the fibroblast cell cluster in ED patients than that in normal patients ($P<0.001$; Fig. 2D). Based on the median expression of OAS1, the fibroblasts were divided into two cell clusters of low and high OAS1 fibroblasts by UMAP (Fig. 2E). The GO, KEGG, and irGSEA analyses among these two cell clusters were detailed in Fig. 2F and H. These results showed that OAS1 mainly played its role in ED via targeting fibroblasts. Finally, by means of integrated MR and scRNA-Seq analyses, we concluded that the positive regulation of OAS1 gene expression could lead to the vicious circle of erectile dysfunction (Fig. 3). As a result, drugs targeting OAS1 in the future might provide more potential opportunities and flexibility for treating ED.

OAS1 (2'-5'-oligoadenylate synthetase 1), had been reported to be significantly involved in various diseases, including tumors and non-tumors. Huffman et al. strongly implicated that OAS1 could serve as an effector gene influencing the severity of COVID-19 via multi-ancestry fine mapping [8]. Yang et al. also pointed out the prognostic and immunological roles of OAS1 in pan-cancer, especially in macrophage M2 polarization and CTL dysfunction [9]. Moreover, OAS1 was found to be not only a significant immune regulatory factor but also significantly associated with regulating biological processes such as apoptosis, cell proliferation, tumorigenesis, and so on [9, 10]. However, the definite roles of OAS1 in ED had never been studied in previous articles. Based on the above-mentioned discussed results, we believed that OAS1 deserved to be studied for its possible functions and clinical values as a drug target in ED.

It was the first time for us to integrate scRNA-Seq and MR analysis with eQTL and pQTL data to identify OAS1 as a novel therapeutic target for ED via targeting fibroblasts. OAS1 mainly showed its superiority in three aspects. Firstly, OAS1 blood-related information could provide useful references for ED oral drug design. Secondly, OAS1 cis-eQTL and cis-pQTL had been shown to exert regulatory effects associated with ED at both the gene and protein levels, providing more potential opportunities and flexibility for treating ED. Finally, therapeutic strategies targeting OAS1 might directly exert negative regulatory effects on ED. Several limitations should also not be ignored. Firstly, since the MR data population were European, it might not be applicable to other study populations. Secondly, while MR and scRNA-Seq analyses were powerful observational tools, experimental validations were crucial to confirm OAS1's role in ED. Lastly, the biological mechanisms underlying the upregulation of OAS1 in fibroblasts contributing to ED needed to be further explored. In conclusion, our study identified OAS1 as a gene of interest in the context of ED via targeting fibroblasts through integrated MR and scRNA-Seq analyses. While these findings highlighted the potential of OAS1 as a therapeutic target, further experimental and clinical studies were still required to validate its functional role and therapeutic relevance in ED pathology.

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Author contributions
Yi Wang & Guihua Chen: Manuscript writing/editing/revision; Yi Wang & Guihua Chen: Data analysis; Yi Wang & Guihua Chen: Data collection or management; Yi Wang & Deng Li: Protocol/project development. All the co-authors agreed to publish the final version of this manuscript.

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Data availability
All of these data were openly accessible. IEU OpenGWAS eQTLs were from the website link https://gwas.mrcieu.ac.uk/; eQTLGen Whole Blood cis-eQTLs were from the website link https://www.eqtlgen.org/cis-eqtls.html; GTEx Whole Blood cis-eQTLs were from the website link https://yanglab.westlake.edu.cn/software/smr/#eQTLsummarydata; Zheng Plasma Protein cis-pQTLs were from the article of Zheng et al. (PMID: 32895551) and erectile dysfunction genetic data were obtained from the website link https://gwas.mrcieu.ac.uk/, with IDs

of ebi-a-GCST006956, including 6,175 cases and 217,630 controls. Single-cell RNA-sequencing data of ED was got from the GSE206528 dataset.

Declarations

Ethics approval and consent to participate
Since the data for this study came from genome-wide association study (GWAS) datasets that were publicly available, ethical approval was therefore not required.

Consent for publication
Not applicable.

Competing interests
The authors declare no competing interests.

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